A new method, using cis-regulatory control, for blocking embryonic gene expression.

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Public Summary:
Most genes, and in particular those genes encoding proteins that regulate the expression of other genes, are used at many times during the development and life of an organism. We have methods to manipulate gene expression at the earliest stages and thus gain insight into the role these genes play. However, it is more challenging to manipulate gene expression only at later stages of life, though this would be critical to do for studies of tissue regeneration. We developed a method to knock down specific genes at virtually any time and place in an organism. We achieve this by using the sequences found in the genome that give a particular gene its “address”, the when and where of its expression. Using these sequences from different “driver genes”, i.e., those that supplied an address, we engineered constructs that would express transcripts that were antisense and therefore would specifically knock down other “target genes”. We used many different combinations of “addresses” and “targets” and determined that this could be a powerful method at virtually any stage of an animal’s life cycle, including in the adult. Employing these or similar tools will therefore likely prove critical for adult tissue regeneration studies.

Scientific Abstract:
Many genes, and particularly regulatory genes, are utilized multiple times in unrelated phases of development. For studies of gene function during embryogenesis, there is often need of a method for interfering with expression only at a specific developmental time or place. Here we show that in sea urchin embryos cis-regulatory control systems which operate only at specific times and places can be used to drive expression of short designed sequences targeting given primary transcripts, thereby effectively taking out the function of the target genes. The active sequences are designed to be complementary to intronic sequences of the primary transcript of the target genes. In this work, the target genes were the transcription factors alx1 and ets1, both required for skeletogenesis, and the regulatory drivers were from the sm30 and tbr genes. The sm30 gene is expressed only after skeletogenic cell ingression. When its regulatory apparatus was used as driver, the alx1 and ets1 repression constructs had the effect of preventing postgastrular skeletogenesis, while not interfering with earlier alx1 and ets1 function in promoting skeletogenic mesenchyme ingression. In contrast, repression constructs using the tbr driver, which is active in blastula stage, block ingression. This method thus provides the opportunity to study regulatory requirements of skeletogenesis after ingression, and may be similarly useful in many other developmental contexts.